

WHAT IS CLAIMED IS:

1. A method for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in a eucaryotic cell, comprising the step of introducing a chimeric DNA comprising the following operably linked parts:

- a) a promoter, operative in said eucaryotic cell;
- b) DNA region, which when transcribed, yields an RNA molecule comprising an RNA region capable of forming an artificial stem-loop structure, wherein one of the annealing RNA sequences of the stem-loop structure comprises a sequence, essentially similar to at least part of the nucleotide sequence of said nucleic acid of interest, and wherein the second of said annealing RNA sequences comprises a sequence essentially similar to at least part of the complement of at least part of said nucleotide sequence of said nucleic acid of interest; and optionally
- c) a DNA region involved in transcription termination and polyadenylation.

2. A method for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in a eucaryotic cell, comprising the step of introducing a chimeric DNA comprising the following operably linked parts:

- a) a promoter, operative in said eucaryotic cell;
- b) a DNA region, which when transcribed, yields an RNA molecule with a nucleotide sequence comprising
 - i. a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of said nucleic acid of interest; and
 - ii. an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence;

wherein the RNA is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence such that at least said 10 consecutive

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- nucleotides of the sense sequence basepair with said 10 consecutive nucleotides of the antisense sequence; and optionally
- c) a DNA region involved in transcription termination and polyadenylation.

- 5 3. The method of claim 2, wherein said RNA molecule further comprises a spacer nucleotide sequence located between said sense and said antisense nucleotide sequence.

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4. The method of claim 2, wherein said sense nucleotide sequence comprises at least about 550 consecutive nucleotides having between 75% and 100% sequence identity with at least part of the nucleotide sequence of said nucleic acid.

5. The method of claim 2, wherein said nucleic acid of interest is a gene integrated in the genome of said eucaryotic cell.

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6. The method of claim 5, wherein said gene is an endogenous gene.

7. The method of claim 5, wherein said gene is a foreign transgene.

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8. The method of claim 2, wherein said chimeric DNA is stably integrated in the genome of the DNA.

9. The method of claim 2, wherein said nucleic acid of interest is comprised in the genome of an infecting virus.

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10. The method of claim 9, wherein said infecting virus is an RNA virus.

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11. The method according to claim 1, wherein said eucaryotic cell is a plant cell.

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12. The method of claim 11, wherein said plant cell is comprised within a plant.

13. A method for reducing the phenotypic expression of a nucleic acid of interest, which is normally capable of being expressed in a eucaryotic cell comprising the step of introducing a chimeric RNA molecule comprising at least one RNA region with a nucleotide sequence comprising

- i. a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of the nucleic acid of interest; and
- ii. an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence;

wherein said RNA region is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence such that at least said 10 consecutive nucleotides of the sense sequence basepair with said 10 consecutive nucleotides of the antisense sequence.

14. A method for reducing the gene expression of a gene of interest in plant cells, said method comprising the step of introducing a first and second chimeric DNA, linked on one recombinant DNA such that both chimeric DNAs are integrated together in the nuclear genome of the transgenic plant cells; wherein said first chimeric DNA comprises the following operably linked parts:

- a) a plant-expressible promoter;
- b) a first DNA region capable of being transcribed into a sense RNA molecule with a nucleotide sequence comprising a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of said gene of interest; and optionally
- c) a DNA region involved in transcription termination and polyadenylation functioning in plant cells; and

wherein said second chimeric DNA comprises the following operably linked parts:

- a) a plant-expressible promoter;
- b) a second DNA region capable of being transcribed into an antisense RNA molecule with a nucleotide sequence comprising an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence; and optionally
- c) a DNA region involved in transcription termination and polyadenylation functioning in plant cells;

wherein said sense and antisense RNA molecules are capable of forming a double stranded RNA by base-pairing between the regions which are complementary.

15. A method for obtaining a virus resistant eucaryotic organism, said method comprising the steps of :

- 1) providing the cells of said organism with a first and second chimeric DNA wherein said first chimeric DNA comprises the following operably linked parts:
 - a) a promoter operative in said cells;
 - b) a first DNA region capable of being transcribed into a sense RNA molecule with a nucleotide sequence comprising a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of the genome of a virus, capable of infecting said organism; and optionally
 - c) a DNA region involved in transcription termination and polyadenylation functioning in said cells; and

wherein said second chimeric DNA comprises the following operably linked parts:

- a) a promoter operative in said cells;
- b) a second DNA region capable of being transcribed into an antisense RNA molecule with a nucleotide sequence comprising an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the

complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence; and optionally

- c) a DNA region involved in transcription termination and polyadenylation functioning in said cells; and

wherein said sense and antisense RNA are capable of forming a double stranded RNA by base-pairing between the regions which are complementary.

16. The method according to claim 15, wherein said organism is a plant

17. The method of claim 16, wherein said cells of said plants are provided with said first and second chimeric DNA by crossing parent plants comprising either said first or said second chimeric DNA.

18. The method of claim 16, wherein said cells of said plants are provided with said first and second chimeric DNA by transforming a plant cell with said first and second chimeric DNA, and regenerating a plant from said transformed plant cell.

19. The method of claim 16, wherein said first and second chimeric DNA are integrated separately in said nuclear genome of said plant cell.

20. The method of claim 16, wherein said first and second chimeric DNA are linked on one recombinant DNA such that both chimeric DNAs are integrated together in the nuclear genome of the transgenic plant cells.

21. A method for identifying a phenotype associated with the expression of a nucleic acid of interest in a eucaryotic cell, said method comprising

- a. selecting within said nucleotide sequence of interest, a target sequence of at least 10 consecutive nucleotides;
- b. designing a sense nucleotide sequence corresponding to the length of the selected target sequence and which has a sequence identity of at least about 75% to about 100% with said selected target sequence;

- c. designing an antisense nucleotide sequence which:
- i) has a sequence identity of at least about 75% to about 100% with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence;
 - ii) comprises a stretch of at least about 10 consecutive nucleotides with 100% sequence identity to the complement of a part of said sense nucleotide sequence;
- d. introducing an RNA molecule comprising both said sense and antisense nucleotide sequences into a suitable eucaryotic host cell comprising the nucleic acid including the nucleotide sequence with hitherto unidentified phenotype; and
- e. observing the phenotype by a suitable method.

22. A eucaryotic cell, comprising a nucleic acid of interest, which is normally capable of being phenotypically expressed, further comprising a chimeric DNA molecule comprising the following operably linked parts:

- a) a promoter, operative in said eucaryotic cell;
- b) a DNA region, which when transcribed, yields an RNA molecule with at least one RNA region with a nucleotide sequence comprising
 - i. a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of the nucleic acid of interest; and
 - ii. an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence;wherein the RNA is capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base-pairing between the regions with sense and antisense nucleotide sequence; and optionally

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c) a DNA region involved in transcription termination and polyadenylation, wherein the phenotypic expression of said nucleic acid of interest is significantly reduced.

23. A eucaryotic cell, comprising a nucleic acid of interest, which is normally capable of being phenotypically expressed, further comprising a chimeric RNA molecule comprising at least one RNA region with a nucleotide sequence comprising

- i. a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of the nucleic acid of interest; and
- ii. an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence;

wherein said RNA is capable of forming an artificial hairpin RNA with a double stranded RNA region by base-pairing between the regions with sense and antisense nucleotide sequence such that at least said 10 consecutive nucleotides of the sense sequence basepair with said 10 consecutive nucleotides of the antisense sequence.

24. A eucaryotic cell, comprising a gene of interest, which is normally capable of being phenotypically expressed, further comprising a first and second chimeric DNA, linked on one recombinant DNA such that both chimeric DNAs are integrated together in the nuclear genome of said eucaryotic cell; wherein said first chimeric DNA comprises the following operably linked parts:

- a) a promoter operative in said eucaryotic cell
- b) a first DNA region capable of being transcribed into a sense RNA molecule with a nucleotide sequence comprising a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of said gene of interest; and optionally

c) a DNA region involved in transcription termination and polyadenylation; and wherein said second chimeric DNA comprises the following operably linked parts:

- a) a promoter operative in said eucaryotic cell;
- b) a second DNA region capable of being transcribed into an antisense RNA molecule with a nucleotide sequence comprising an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence; and optionally

c) a DNA region involved in transcription termination and polyadenylation; wherein said sense and antisense RNA molecules are capable of forming a double stranded RNA region by base-pairing between the regions which are complementary.

25. The eucaryotic cell of claim 22, which is a plant cell.

26. A plant comprising the plant cell of claim 25.

27. A virus resistant plant, comprising a first and second chimeric DNA integrated in the nuclear genome its cells, wherein said first chimeric DNA comprises the following operably linked parts:

- a) a plant-expressible promoter;
- b) a first DNA region capable of being transcribed into a sense RNA molecule with a nucleotide sequence comprising a sense nucleotide sequence of at least 10 consecutive nucleotides having between 75 and 100% sequence identity with at least part of the nucleotide sequence of the genome of a virus, capable of infecting said plant; and optionally
- c) a DNA region involved in transcription termination and polyadenylation functioning in plant cells; and

wherein said second chimeric DNA comprises the following operably linked parts:

- a) a plant-expressible promoter;

b) a second DNA region capable of being transcribed into an antisense RNA molecule with a nucleotide sequence comprising an antisense nucleotide sequence including at least 10 consecutive nucleotides, having between about 75% to about 100% sequence identity with the complement of said at least 10 consecutive nucleotides of said sense nucleotide sequence;

c) optionally, a DNA region involved in transcription termination and polyadenylation functioning in plant cells; and

wherein said sense and antisense RNA are capable of forming a double stranded RNA region by base-pairing between the regions which are complementary.

28. The plant of claim 27, wherein said first and second chimeric DNA are integrated in one locus in the nuclear genome.

29. The plant of claim 27, wherein said first and second chimeric DNA are integrated in different loci in the nuclear genome.

30. A method for modifying the fatty acid profile in oil from a plant, said method comprising the step of introducing a chimeric DNA into the cells of said plant, said chimeric DNA comprising the following operably linked parts:

- a). a plant-expressible promoter
- b). a DNA region, which when transcribed yields an RNA molecule comprising an RNA region capable of forming an artificial stem-loop structure, wherein one of the annealing RNA sequences of the stem-loop structure comprises a nucleotide sequence essentially similar to at least part of the nucleotide sequence of a $\Delta 12$ desaturase encoding open reading frame, and wherein the second of said annealing RNA sequences comprises a sequence essentially similar to at least part of the complement of at least part of the nucleotide sequence of said $\Delta 12$ desaturase encoding open reading frame; and optionally;
- c) a DNA region involved in transcription termination and polyadenylation.

31. The method of claim 30, wherein said DNA region comprises the nucleotide sequence of SEQ ID No 6.

5 32. The method of claim 30, wherein said modifying of the fatty acid profile, comprises increasing the oleic acid content.

33. The method of claim 30, wherein said plant expressible promoter is a seed-specific promoter.

10 34. The method of claim 30, wherein said plant is oilseed rape.

35. A plant producing oil with modified fatty acid profile, said plant comprising a chimeric DNA, said chimeric DNA comprising the following operably linked parts:

- 15 a). a plant-expressible promoter
- b). a DNA region, which when transcribed yields an RNA molecule comprising an RNA region capable of forming an artificial stem-loop structure, wherein one of the annealing RNA sequences of the stem-loop structure comprises a nucleotide sequence essentially similar to at least part of the nucleotide sequence of a $\Delta 12$ desaturase encoding open reading frame, and wherein the second of said annealing RNA sequences comprises a sequence essentially similar to at least part of the complement of at least part of the nucleotide sequence of said $\Delta 12$ desaturase encoding open reading frame; and optionally;
- 20 c). a DNA region involved in transcription termination and polyadenylation.

25 36. The plant of claim 35, wherein said DNA region comprises the nucleotide sequence of SEQ ID No 6.

30 37. The plant of claim 35, wherein said plant expressible promoter is a seed-specific promoter.

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